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DURABILITY OF SKIN EXPOSURE REDUCTION PASTE AGAINST CHEMICAL WARFARE AGENTS (SERPACWA) WHEN WORN UNDER PROTECTIVE CLOTHING

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L.A. Stephenson, W.A. Latzka, M.J. McCreery, L. Levine, S. B. Robinson, C.M. Kesick, M.A. Kolka

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Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), previously known as Topical Skin Protectant (TSP), was developed to augment the protection afforded by chemical protective clothing. Although the Food and Drug Administration (FDA) approved the New Drug Application (NDA #21-084) as a safe and effective topical drug for its intended use, the FDA and the U.S. Army Combat Developers requested additional studies to determine the specific guidance required to optimize the field use of SERPACWA.

The purpose of the current experiments was twofold: 1) to determine the degree of protection that SERPACWA provided against a vasodilatory challenge at 8, 16, 12 and 24 h of wear; and 2) to determine when SERPACWA should be re-applied to the skin to provide optimal protection. SERPACWA was applied (50 uL/site) to skin sites on one, randomly chosen arm, while no SERPACWA was applied to the other arm. One wrist site on each arm was not challenged with the vasodilatory challenge, methyl nicotinate (Mnic). Three remaining sites on each arm received a 2-min challenge with 10 µl/site of 5.0 mMol Mnic. Laser-Doppler Imaging (LDI) scans and visual scores were performed prior to SERPACWA application and approximately 15 min post-Mnic challenge after 8, 12, 16, 24 h, and after 24 h when SERPACWA was re-applied to the skin test site at 8 h.

This study (Experiments III and IV) provides clear and convincing evidence that SERPACWA possesses significant skin protection when worn under field-relevant garments for 8, 12 and 16 h. SERPACWA provided complete protection at 8 h, 66% protection at 12 h and 60% protection at 16 h. The test to determine protection at 24 h was inconclusive according to the LDI data. SERPACWA provided 58% protection when it was re-applied after 8 h of wear and tested at 24 h of wear. The visual score data in general corroborate the LDI data with the exception that visual scores indicated that SERPACWA worn for 24 h did provide protection against the challenge. The presence of a film of SERPACWA having an approximate thickness of only 0.1 mm very significantly reduced skin exposure to Mnic challenges that otherwise induce significant vasodilation.

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DURABILITY OF SKIN EXPOSURE REDUCTION PASTE AGAINST CHEMICAL WARFARE AGENTS (SERPACWA) WHEN WORN UNDER PROTECTIVE CLOTHING

Lou A. Stephenson
William A. Latzka
Michael J. McCreery
Leslie Levine
Scott B. Robinson
Christina M. Kesick
Margaret A. Kolka

Thermal and Mountain Medicine Division

November 2001

U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

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EXECUTIVE SUMMARY

Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), previously known as Topical Skin Protectant (TSP), was developed to augment the protection afforded by chemical protective clothing. SERPACWA, applied to the skin at the closure sites of chemical protective clothing, may provide additional protection against the percutaneous threat of chemical agents. Although the Food and Drug Administration (FDA) approved the New Drug Application (NDA #21-084) as a safe and effective topical drug for its intended use, the FDA and the U.S. Army Combat Developers requested additional studies to determine the specific guidance required to optimize the field use of SERPACWA. In this paper, we report the outcome of the third and fourth of four experiments conducted at The U.S. Army Research Institute of Environmental Medicine (USARIEM), at the request of the U.S. Army Medical Materiel Development Activity (USAMMDA), to determine the durability of SERPACWA after it was worn under the battle dress uniform (BDU) and a chemical protective jacket.

The purpose of the current experiments was twofold: (1) to determine the degree of protection that SERPACWA provided against a vasodilatory challenge at 8, 16, 12 and 24 h of wear; and (2) to determine when SERPACWA should be re-applied to the skin to provide optimal protection. Either two or four 2.4 cm diameter circular sites were marked on each arm of six subjects for each of the five tests. SERPACWA was applied (50 µL/site) to skin sites on one randomly chosen arm, while no SERPACWA was applied to the other arm. One wrist site on each arm was not challenged with the vasodilatory challenge, methyl nicotinate (Mnic). Three remaining sites on each arm received a 2 min challenge with 10 µl/site of 5.0 mMol Mnic. Laser-Doppler Imaging (LDI) scans and visual scores were performed prior to SERPACWA application and approximately 15 min post-Mnic challenge after 8, 12, 16, 24 h, and after 24 h when SERPACWA was re-applied to the skin test site at 8 h.

Results from this study (Experiments III and IV) provide clear and convincing evidence that SERPACWA possesses significant skin protection when worn under field-relevant garments for 8, 12 and 16 h. Based on percentage change in perfusion as measured by LDI (identified as the primary measure in the protocol), SERPACWA provided complete protection at 8 h, 66% protection at 12 h, and 60% protection at 16 h. The test to determine protection at 24 h was inconclusive according to the LDI data. SERPACWA provided 58% protection when it was re-applied after 8 h of wear and tested at 24 h of wear. The visual score data, in general, corroborate with the LDI data, with the exception that visual scores indicated that SERPACWA worn for 24 h did provide partial protection against the challenge. The presence of a film of SERPACWA having an approximate thickness of only 0.1 mm very significantly reduced skin exposure to Mnic challenges that otherwise induce significant vasodilation.

INTRODUCTION

Chemical warfare agents (CWA) continue to pose a threat to U.S. warfighters and peacekeepers. Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), previously known as Topical Skin Protectant (TSP), has been proposed to compliment the use of chemical protective clothing and gear as additional protection against the percutaneous threat of CWA at the closure sites of these garments (6). It is non-reactive, non-wetting and immiscible with most other chemicals. SERPACWA is a thick cream that can be spread in a thin, even layer on the skin, comprised of a Teflon®-like mixture in perfluoroalkylpolyether, which creates an inert, passive physical barrier over the stratum corneum (19, 20). This barrier prevents penetration and percutaneous absorption of a wide variety of substances including chemical or biological warfare agents. It is non-irritating to skin and eyes and can be used over a wide range of temperatures (19, 20, 30). These characteristics make it an ideal candidate for preventing skin contact with CWA.

SERPACWA studies have included the use of a skin challenge agent such as an allergen or toxin. Urushiol (poison ivy) extract and methyl nicotinate (Mnic) have been used as challenge agents in human subject studies to test SERPACWA's efficacy (2, 6, 30). These challenge agents cause skin erythema and vesiculation following unprotected skin exposure. SERPACWA treated skin was protected against both of these challenge agents (3, 7). Using Mnic as the challenge agent, SERPACWA was shown to be an effective skin protectant for up to an hour in sweating humans (6). The rapid skin response to Mnic (non-immunologic contact reaction), manifested by erythema or urtication, generally within minutes, makes it useful as a challenge agent.

After the Food and Drug Administration (FDA) approved the New Drug Application (NDA) for SERPACWA (NDA #21-084) as a safe and effective topical drug for its intended use, the FDA and the Army Combat Developers requested additional studies to determine the specific guidance required to optimize the field use of SERPACWA. In this paper we report the outcome of the third and fourth of four experiments conducted at The U.S. Army Research Institute of Environmental Medicine (USARIEM), at the request of the U.S. Army Medical Materiel Development Activity (USAMMDA), to determine the durability of SERPACWA after it was worn under the BDU and a chemical protective jacket. Previous reports addressed the determination of challenge agent concentration (13) and whether prior treatment with isopropyl alcohol was necessary for SERPACWA efficacy (18).

The purpose of the current experiments was twofold: 1) to determine the degree of protection that SERPACWA provided against a vasodilatory challenge at 8, 12, 16 and 24 h of wear; and 2) to determine when SERPACWA should be re-applied to the skin to provide optimal protection.

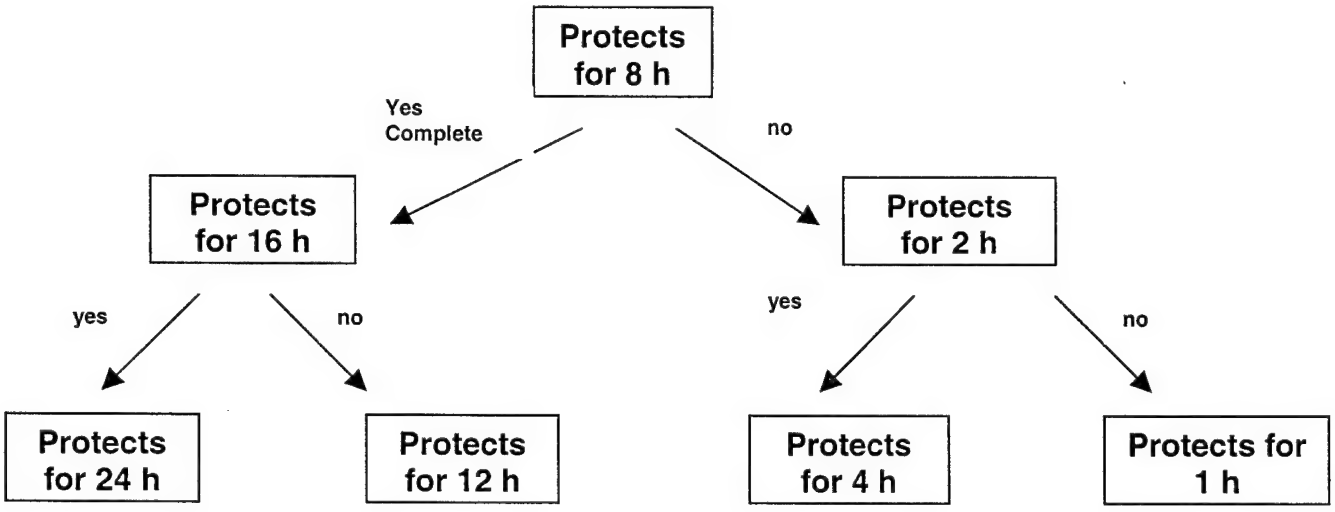
METHODS

This research study was conducted in compliance with applicable Good Clinical Practice regulations, with USARIEM Standard Operating Procedures, and as described in the study protocol (17).

STUDY DESIGN

Figure 1 shows the experimental plan to determine the wear-time or durability for which SERPACWA provides protection against the challenge agent in Experiment III.

Figure 1. Scheme showing SERPACWA durability evaluation.



Experiment III

Categories of protection were defined as follows:

Complete protection: Complete protection was defined as the absence of a significant difference in flux values between the treated/challenged and the treated/unchallenged sites, so long as there was a significant difference between both of these sites and the untreated/challenged control.

Significant protection: Protection was defined as significant if the flux of the treated/challenged site was significantly different from both the untreated/challenged site and the treated/unchallenged site.

No protection: The results for a specific wear-time trial were defined as affording no protection if the treated/challenged site was not statistically different from the untreated/challenged site, but both of these sites are statistically distinct from the treated/unchallenged site.

No conclusion: The results for a specific wear-time trial were defined as inconclusive if the treated/challenged site was not statistically different from the untreated/challenged site, and from the treated/unchallenged site.

SERPACWA provided complete protection from Mnic challenge for the first wear-time trial of 8 h, so the next trial employed a 16 h wear time. By following the wear-time trial strategy outlined in the scheme shown in Figure 1, the wear time for complete protection was established. After this time period was known, then the same bracketing approach was used to establish the wear time for significant protection, starting with the next wear-time period in which SERPACWA failed to provide complete but provided at least significant protection. For example, if significant protection were observed at 16 hours, then the next trial would have been for a 24 hour wear time. Trials were concluded when wear times for both complete and significant protection were defined and when the upper limit of trial times (24 h) was tested.

The sequence of conducting tests in Experiment III followed the plan in Figure 1. Test 1 was conducted on 25 Jan 01. SERPACWA durability was tested for 8 h, with the conclusion of the test (final measurements made) occurring at approximately 1700 h. Test 2 was conducted for 16 h on 29-30 Jan 01, with the conclusion of the test occurring at approximately 0200 h. Test 3 was conducted for 12 h on 01 Feb 01 with the conclusion of the test occurring at approximately 2130 h. Test 4 was conducted on 21-22 June 2001. Test 4 concluded at approximately 1045 h.

Experiment IV was conducted for 24 h on 5-6 February 01. Experiment IV concluded at approximately 1400 h.

Six volunteers were studied in each test conducted in Experiments III (Tests 1-4) and IV (one test). Originally, the sample size was estimated by power analysis for a paired t-test using standard deviations derived from LDI flux measurements (96 and 73 for untreated and treated sites, respectively) from a previous SERPACWA study (6). The sample size was estimated using an alpha = 0.05 and power = 0.80. For the last test conducted (Experiment III, Test 4), sample size was estimated by power analysis for an ANOVA design based on the data collected in Experiment III, Test 3.

Test Subject Selection

A total of 14 healthy soldiers assigned to the Natick Soldier's Systems Center participated as subjects for this research. Each was fully informed of all test procedures

and risks, then read and signed an approved, informed consent document. Table 1 shows subject characteristics for each test. After giving written consent, the subjects were cleared by the medical monitor and screened with respect to the inclusion/exclusion criteria (listed below).

Inclusion Criteria – Before acceptance as test subjects, volunteers had to meet the following criteria:

1. Volunteers were active duty military; unrestricted as to race, ethnicity or gender; 18-55 years of age; and generally in good health, as established through medical examination.
2. Volunteers had a resting blood pressure no greater than 140/90 and a resting pulse rate of 50-100 bpm.
3. Female volunteers had a negative urine pregnancy test at enrollment.
4. Volunteers were willing to abide by the rules of the study.
5. Volunteers' volar forearm and wrists were free of scars, tattoos, cuts or abrasions that would interfere with test measurements, and the width of the volar surface exceeded 5.5 cm as measured at the wrist.
6. Volunteers signed the informed consent document.
7. Volunteers were willing and able to refrain from alcohol intake for 24 hours prior to the start of each day of testing.
8. Volunteers were willing and able to refrain from using any medications (prescription or over-the-counter) except for oral contraceptives for 2 days prior to testing, until testing was completed. These included drugs classified as antihistamines, anti-inflammatories including corticosteroids, cortisone, aspirin, ibuprofen, and non-steroidal anti-inflammatories, nicotine or other transdermal delivery patches, diet pills or other medications or dietary supplements which might interfere with test evaluations.
9. Volunteers had an erythemic response to a topical application of (10ul, 2.5 mM) methyl nicotinate.
10. Volunteers had a current (within a year) physical exam.

Exclusion Criteria – Volunteers were not allowed to participate as test subjects if any of the following existed:

1. Female volunteer was pregnant or breast-feeding.
2. Volunteer had a skin disorder or condition that would interfere with test evaluations (e.g., eczema, psoriasis, atopic dermatitis, sunburn, significant tanning).
3. Volunteer had a history of chronic or systemic disease including rheumatoid arthritis or other inflammatory disorders, diabetes, high blood pressure, history of epilepsy, severe asthma, or any medical condition that might interfere with cutaneous vasodilation or inflammation.
4. Volunteer was using medication on a regular basis such as antihistamines, insulin, anti-inflammatory agents including corticosteroids, cortisone containing preparations, aspirin, ibuprofen, and non-steroidal anti-inflammatories, nicotine or other transdermal delivery patches, diet pills or other medications or dietary supplements which might interfere with test evaluations.
5. Volunteer had a known allergy or sensitivity to one or more components of test materials including adhesives and latex.
6. Volunteer was a smoker.
7. Volunteer was a moderate or heavy drinker who would not likely be able to refrain from alcohol consumption for 24 hours prior to testing.
8. Volunteer did not have a normal reaction to topical application of methyl nicotinate.

9. Volunteer reacted to skin application of SERPACWA.

During this clearance process, to test the volunteers for any unusual reaction to SERPACWA, one of the study investigators trained in SERPACWA application (per study SOP) applied 50 ul of SERPACWA to a 2.4 cm diameter site on the volar surface of each volunteer's forearm and removed it after 10 min. To test the volunteers for reactivity to Mnic, 10 ul of 2.5 mM Mnic was applied to another site on each volunteer's forearm and was removed in 2 min. Both investigator and physician observed the skin reactions to these products at 15, 30 and 60 min post-application. Each volunteer was admitted to the study as a test subject when the physician cleared him/her, and when he/she met/did-not-meet the inclusion/exclusion criteria.

Forearm Treatment Sites

Prior to testing, a black felt template was made for each subject and was used as a stencil for defining and marking the test sites on the volar surface of the forearms near the wrist. Four 2.4 cm circular test sites (two on each forearm, spaced approximately 1 cm apart), free of scars, blemishes or tattoos, were identified with a skin-safe marking pen. The template was designed so that the test sites' distal boundary began about 2 cm proximal to the wrist crease. The template was also placed on the forearms prior to initiating each LDI scan to provide contrast for LDI flux graphic display. Each site on a SERPACWA-treated arm had a control site (no SERPACWA treatment) at the same relative position on the contra lateral forearm. The arm selected for SERPACWA treatment (right or left) was randomly assigned. Table 1 tabulates the arm selected for SERPACWA application for the tests in Experiments III and IV.

Two or four 2.4 cm diameter circular sites were identified on the volar surface of each forearm for each test subject. Figure 2 shows the skin test sites for Experiment III, Test 1 when there were four sites per arm. When there were two sites, the sites used were the distal sites (near the wrist). After the test sites were marked on the forearm, SERPACWA was applied in a band on the wrist as described below. After the allotted time to test for durability, three of the test sites on each arm received a 2-min challenge with Mnic. LDI scans were performed prior to the application of SERPACWA (Scan 1), and approximately 15 min post-Mnic challenge (Scan 2).

SERPACWA Application

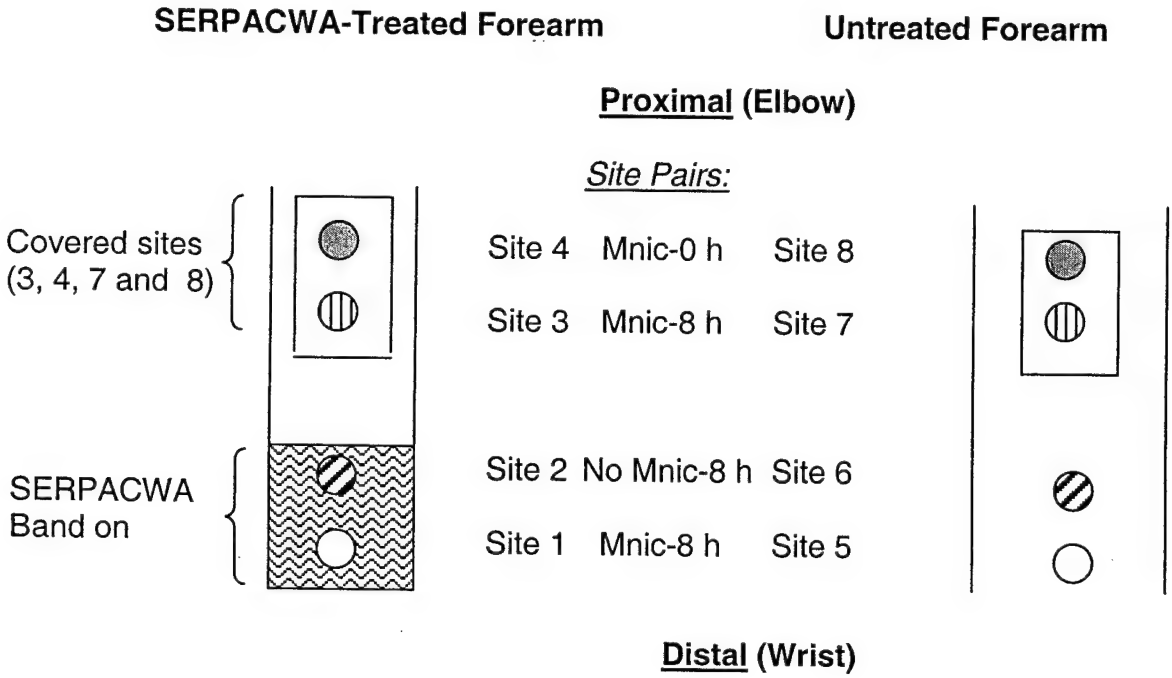
SERPACWA was applied (0.153 g SERPACWA per cm of wrist circumference) on a randomly chosen wrist in a 7 cm wide band around the wrist. An Investigator using only the index finger of a gloved hand rubbed SERPACWA on the volunteer's skin for 2 minutes. SERPACWA was not applied to the other wrist. At the two wrist test sites within the SERPACWA-treated area, the SERPACWA was removed and reapplied more exactly by delivery of 11 uL/ cm^2 of SERPACWA to the center of each test site and then spread evenly over that site. This volume was calculated to provide 0.1 mm layer of SERPACWA on the skin. For the two sites that were above the wrist, SERPACWA was applied as previously described for Experiment II (18). The Sponsor specified this dose to maintain consistency with prior SERPACWA efficacy testing

Table 1. Subject characteristics for each test done in Experiments III and IV.

Exp/Test	Subject	Gender	Age (yrs)	Ethnicity	Height (m)	Weight (kg)	Handedness/ Application arm
III / 1							
	11	M	18	H	1.74	83.8	R/L
	12	M	20	C	1.80	92.8	L/L
	14	M	19	C	1.85	87.8	R/R
	15	M	19	AA	1.88	91.1	R/L
	16	M	20	C	1.70	73.5	R/L
	19	M	19	C	1.61	78.6	R/R
	Mean		**19.2**		**1.76**	**84.6**	
	SD		**0.8**		**0.10**	**7.5**	
III / 2							
	11	M	18	H	1.74	83.8	R/L
	12	M	20	C	1.80	92.8	L/L
	13	F	18	AA	1.57	56.8	R/R
	14	M	19	C	1.85	87.8	R/R
	15	M	19	AA	1.88	91.1	R/L
	16	M	20	C	1.70	73.5	R/L
	Mean		**19.0**		**1.76**	**81.0**	
	SD		**0.9**		**0.11**	**13.7**	
III / 3							
	11	M	18	H	1.74	83.8	R/L
	12	M	20	C	1.80	92.8	L/L
	14	M	19	C	1.85	87.8	R/R
	15	M	19	AA	1.88	91.1	R/L
	16	M	20	C	1.70	73.5	R/L
	19	M	19	C	1.61	78.6	R/R
	Mean		**19.2**		**1.76**	**84.6**	
	SD		**0.8**		**0.10**	**7.5**	
III / 4							
	7	F	21	AA	1.55	67.3	R/R
	20	M	18	C	1.81	96.8	R/R
	21	M	28	AA	1.83	87.1	L/L
	22	F	23	AA	1.58	58.1	R/L
	23	F	19	AA	1.61	68.0	R/R
	24	M	18	C	1.76	83.0	R/L
	Mean		**21.2**		**1.69**	**76.7**	
	SD		**3.9**		**0.12**	**14.6**	
IV							
	12	M	20	C	1.80	92.8	L/L
	13	F	18	AA	1.57	56.8	R/R
	14	M	19	C	1.85	87.8	R/R
	16	M	20	C	1.70	73.5	R/L
	17	M	26	C	1.70	78.2	R/R
	19	M	19	C	1.61	78.6	R/R
	Mean		**20.3**		**1.71**	**78.0**	
	SD		**2.9**		**0.11**	**12.5**	

on animals and humans (6). This dosage allows for a 10% loss due to potential wastage during application; the net dose being approximately 10 ul/cm^2. A Teflon®-coated spatula was used to spread the SERPACWA evenly over the area for a thickness of approximately 0.1 mm. Before the last LDI scan, the SERPACWA was removed with a small, flat, dull blade, Teflon®-coated spatula. After visual scoring was done, the subjects' arms were washed with warm water and soap to remove any remaining SERPACWA.

Figure 2. Diagram for site pairs (Experiment III, Test 1).



After the pre-SERPACWA control LDI scan, SERPACWA was applied to three of the four sites on the randomly chosen SERPACWA-treated arm (Figure 2). In the following narrative, we describe the procedures and sites as if SERPACWA was applied to the right arm as shown in Figure 2. (If SERPACWA were applied to the left arm, the site numbers would change accordingly.) SERPACWA was applied in a 7 cm wide band around the wrist. After SERPACWA was rubbed in, the SERPACWA was removed from the test sites on the wrist (sites 1 and 2) using a spatula. SERPACWA was re-applied using a more exacting technique (described below) to ensure the thickness was correct on sites 1 and 2, and on one of either site 3 or 4, which was randomly selected. After SERPACWA was applied, the study staff dressed the subject in the BDU shirt and the JSLIST chemical protective jacket securing the sleeves of the

BDU and the jacket. After dressing, the subject performed various low intensity activities and Skill Level 1 Common Task Training while wearing the chemical protective jacket. The tasks included performing first aid, donning protective gear, maintenance of the protective mask and recreational games (cards, darts, and other board games). The 8, 12, 16 and 24 h tests all included the same light activities and appropriate meals; the 24 h test included normal sleep time. After the set wear time for that test, SERPACWA was applied to the untreated site (either 3 or 4) of the SERPACWA treated arm. The Mnic challenge was applied at the set time (8, 12, 16, or 24 h) to all contra-lateral pair sites except for either 1 and 5, or 2 and 6, which was randomly selected. The unchallenged sites were the control sites to ascertain differences in blood flow over time.

Methyl Nicotinate Preparation and Application

Methyl nicotinate (≥99% purity, Sigma Aldrich Chemicals) was used as the challenge agent in this study. Methyl nicotinate (methyl 3-pyridinecarboxylate), a lipid soluble ester of nicotinic acid, is a well-studied contact irritant, producing easily monitored reactions. The non-immunologic contact reaction is due to increased prostaglandin, an inflammatory mediator released after Mnic penetration through the stratum corneum into the dermis (3, 4, 12, 5, 25, 28, 31). The exact dosage and timing of the Mnic challenge used in this study, 10 ul of 5 mM Mnic for 2 min, were determined in the first experiment (13) conducted under this study protocol (17). That experiment was conducted to determine the best dose and timing of Mnic to be used for the remaining experiments. A 2 min exposure of 5 mM Mnic resulted in an erythema that was visible in less than 10 min and increased in intensity to peak at 12-22 min post-challenge, and gradually decreased thereafter. The majority of healthy adults have this response to Mnic (the inclusion criteria for volunteer clearance in this study was a visible reaction to 2.5 mM), which is otherwise innocuous at the dilute concentrations and limited duration of exposure employed in this study. The Mnic stock solution (50 mM in distilled water) was prepared from the crystalline solid each test day. The 5 mM test solution was also prepared from the stock solution each test day. At the end of the period of SERPACWA wear; a 10-ul volume of the 5 mM Mnic solution was applied to each of the test sites requiring Mnic challenge. Mnic was removed 2 min after its application by use of a cotton swab to wick the droplets off each test site, followed by blotting with a cotton gauze square.

Scanning Laser Doppler Imagery

Flux measurements by laser Doppler imaging (LDI) and visual scoring have been routinely used to assess erythema (6, 7, 16, 30). LDI provides a sensitive, accurate, reproducible and noninvasive means of measuring changes in skin blood flow (8, 9, 12, 15, 16, 25). The LDI scanning technique used in this study (Moor Industries, Inc., Scanning LDI Unit) provides a 2-dimensional pattern of microcirculation, and produces a

visual image and quantification of perfusion intensity and area. The LDI scans were used to determine the skin response to Mnic, assess the efficacy of SERPACWA, and assess the effect of the alcohol pre-treatment. This LDI technique provides less variance than single point technology and has the ability to evaluate several test sites in a single scan (24). The technique has been validated by numerous studies and has been found to be highly reliable in assessing changes in blood flow resulting from cutaneous exposure to Mnic (1, 5, 11, 14, 16, 21, 23, 24, 27, 31). In addition, the changes in blood flow reported by LDI have correlated well with erythema; the LDI data can be corroborated with visual scoring (1, 15), as was performed in this study. The validity and reliability of LDI have also been demonstrated in the Phase 3 clinical investigation "The Protective Efficacy of the Topical Skin Protectant (TSP) Against Methyl Nicotinate Under Sweating Conditions" (6). That investigation was sponsored by the U.S. Army (USAMMDA) and was presented as one of two pivotal studies in support of the NDA submitted to and approved by the FDA.

For each scan, subjects placed their forearms in a custom made rest that positioned their hands in supination with the forearms and wrists close together, directing the volar test surfaces upward, toward the laser. Subjects wore laser eye protective glasses during the scans. All test sites were scanned for baseline measurements before treatment.

Visual Evaluation

The visual evaluation was used as a secondary assessment of skin reaction to Mnic and was used to corroborate the primary endpoint from the LDI flux measurements. Visual evaluation has been shown to correlate with LDI flux data when exposure to Mnic was assessed (1, 16). The technique originally used a 5-point scale from 0 to 4 and was first reported as a means to assess skin lesions (7). Since its introduction and modification to include half integers, visual evaluations have been widely used as a clinical and research tool (1, 10). In addition, visual evaluation is an FDA requirement in Phase 1 studies for all topically active pharmaceuticals and biologics under Investigational New Drug clinical investigation. Visual evaluations were also used in the two clinical studies pivotal to the FDA approval of the NDA for SERPACWA: the "Sweat Study" (6) and the "Poison Ivy Studies" (29, 30). Visual scores in the "Sweat Study", used to corroborate LDI flux data, correlated with all levels of erythema from Mnic exposure (6). In the current study, the visual evaluations of all test sites were done prior to SERPACWA application (baseline), and following the LDI flux measurements after the Mnic challenge. An experienced evaluator used a 7-point scale from 0 to 3 (6), with intermediate scores of 0.5 used at the evaluator's discretion, defined as follows:

0 = no reaction, no erythema
1 = mild reaction, minimal macular erythema, faint but definitely pink
2 = moderate reaction, moderate macular erythema, definite redness, possible edema
3 = strong to severe reaction, intense redness, definite edema, possible spreading

In several cases, the evaluator was not definitive in his use of intermediate scores. In that case the score was judged to be a quarter point different from either a whole or intermediate score.

STATISTICAL ANALYSES

A one-way repeated measures analysis of variance was used to compare the post-Mnic challenge data in Experiment III (Tests 1-4) and Experiment IV. The comparisons made were to determine: (1) whether or not SERPACWA application protected the skin against Mnic challenge for the specific wear-times (8, 12, or 16 h), LDI flux and visual scoring the SERPACWA Mnic-challenged site was compared to the site that had no SERPACWA, but was Mnic-challenged; (2) whether or not SERPACWA protected the skin completely, the SERPACWA-treated, and Mnic-challenged site was compared to the SERPACWA-treated, no Mnic challenge site; and (3) whether SERPACWA itself affected SkBF, the SERPACWA-treated, but no Mnic challenge site was compared to the contralateral site that was not treated with either SERPACWA or Mnic. The Mnic-only site served as a test of validity that the skin responded to the Mnic challenge. As reported in the Test Subject Selection section, all volunteers were screened for Mnic responsiveness; only responders were included in further testing. For all comparisons, significance was accepted at the 95% confidence level ($p<0.05$).

RESULTS

The flux and visual score data for the five tests in Experiments III and IV are tabulated in Tables 2 – 6. For Experiment III, Test 1, SERPACWA treatment provided complete protection against the Mnic challenge after 8 h of wear as shown by the lack of difference in flux between the SERPACWA-treated, Mnic-challenged site and the SERPACWA-treated, no Mnic challenge site (Table 2). There was a significant difference between the SERPACWA-treated, Mnic-challenged site and the no SERPACWA treatment, Mnic-challenged site ($P \leq 0.001$, Table 2). The same differences were apparent in the visual scores (Table 2). Figure 3 shows the percentage change in flux from the baseline to post-Mnic application LDI scan and for the visual scores that were made immediately following each scan.

For Experiment III, Test 2 SERPACWA treatment provided significant protection against Mnic challenge after 16 h of wear as shown by a significant difference in flux between the SERPACWA-treated, Mnic-challenged site and the no SERPACWA treatment, Mnic-challenged site ($P \leq 0.001$, Table 3). However, there was also a significant difference in flux between the SERPACWA-treated, Mnic-challenged site and the SERPACWA-treated, no Mnic challenge site ($P \leq 0.001$). This significant difference indicated that the protection provided by SERPACWA was not complete after 16 h of wear. The calculated protection as a percentage change in flux was 60%, as shown in Figure 4. The visual scores that were made immediately following each scan showed

similar results as the flux data (Table 3). Figure 4 also shows the change in visual scores.

Table 2. Skin perfusion (LDI) and visual score (VS) data for each subject for Experiment III, Test 1 (8h). Data listed under the headings LDI Pre and VS Pre was determined before application of SERPACWA. Data listed under the headings LDI Post and VS Post was determined after 8 h of wear. Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. #NSC > SC, SNC & NSNC and SC=NSC=NSNC ($P \leq 0.001$); *NSC > SC ($P \leq 0.001$); and †SC > SNC & NSNC ($P \leq 0.001$).

LDI Pre

Subject #	SC	NSC	SNC	NSNC
11	75.9	94.5	49.3	45.4
12	72.9	84.2	76.6	57.7
14	68.0	72.9	56.9	70.8
15	39.6	35.9	43.7	35.2
16	114.9	64.1	71.6	59.3
19	47.3	70.3	52.8	61.8
Mean	**69.8**	**70.3**	**58.5**	**55.0**
SD	**26.5**	**20.0**	**12.9**	**12.7**

LDI Post

Subject #	SC	NSC	SNC	NSNC
11	62.6	105.5	47.6	67.3
12	179.8	295.5	144.5	99.7
14	124.2	294.2	61.1	73.7
15	61.1	130.8	20.7	31.5
16	148.7	190.7	58.9	54.6
19	96.3	310.3	70.3	61.6
Mean	**112.1**	**221.2#**	**67.2**	**64.7**
SD	**47.7**	**90.9**	**41.6**	**22.5**

VS Pre

Subject #	SC	NSC	SNC	NSNC
11	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0
Mean	**0.0**	**0.0**	**0.0**	**0.0**
SD	**0.0**	**0.0**	**0.0**	**0.0**

VS Post

Subject #	SC	NSC	SNC	NSNC
11	0.5	1.0	0.0	0.0
12	0.5	1.5	0.0	0.0
14	0.5	2.0	0.0	0.0
15	0.5	1.5	0.0	0.0
16	1.0	1.5	0.0	0.0
19	0.5	2.5	0.0	0.0
Mean	**0.6***	**1.7**	**0.0†**	**0.0†**
SD	**0.2**	**0.5**	**0.0**	**0.0**

Figure 3. The mean (±SD) percentage change in flux from LDI scans at baseline and after Mnic challenge (right ordinate) and for the visual scores after Mnic challenge (left ordinate) for Experiment III, Test 1 (8 h). Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. # NSC >SC, NSC, NSNC and SC = NSC, NSNC ($P \leq 0.001$); * NSC > SC ($P \leq 0.001$); † SC > NSC, NSNC ($P \leq 0.001$).

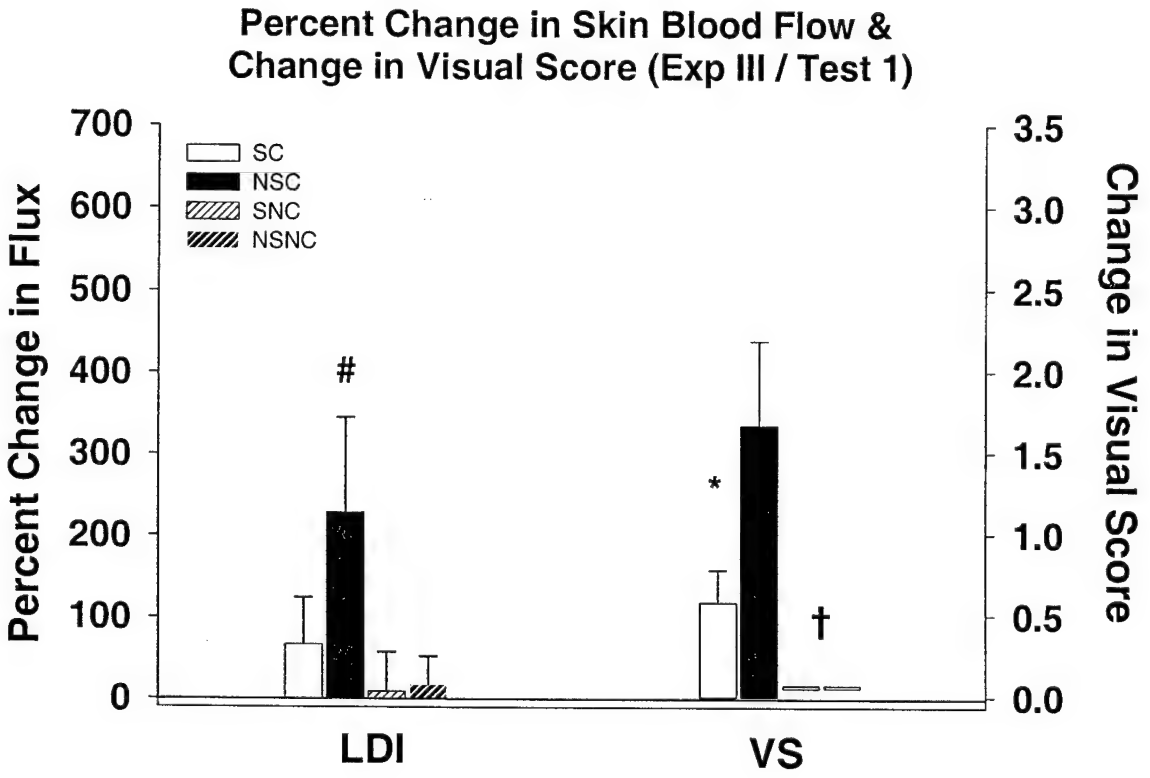


Table 3. Skin perfusion (LDI) and visual score (VS) data for each subject for Experiment III, Test 2 (16 h). Data listed under the headings LDI Pre and VS Pre was determined before application of SERPACWA. Data listed under the headings LDI Post and VS Post was determined after 16 h of wear. Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. *NSC > SC ($P \leq 0.001$); and †SC > SNC & NSNC ($P \leq 0.001$).

LDI Pre

Subject #	SC	NSC	SNC	NSNC
11	58.5	86.6	40.1	98.0
12	156.1	78.8	79.1	62.5
13	32.9	31.5	35.2	34.9
14	49.1	64.2	68.1	88.6
15	38.2	57.2	41.1	66.8
16	86.1	57.6	62.6	73.3
Mean	**70.2**	**62.7**	**54.4**	**70.7**
SD	**46.1**	**19.3**	**18.0**	**22.1**

LDI POST

Subject #	SC	NSC	SNC	NSNC
11	125.7	202.9	63.9	52.8
12	188.0	342.8	74.6	99.4
13	85.4	113.1	35.8	39.8
14	65.9	271.2	53.5	93.3
15	160.8	301.2	52.1	121.0
16	165.5	276.2	61.6	67.4
Mean	**131.9***	**251.2**	**56.9†**	**79.0†**
SD	**48.3**	**81.6**	**13.1**	**30.8**

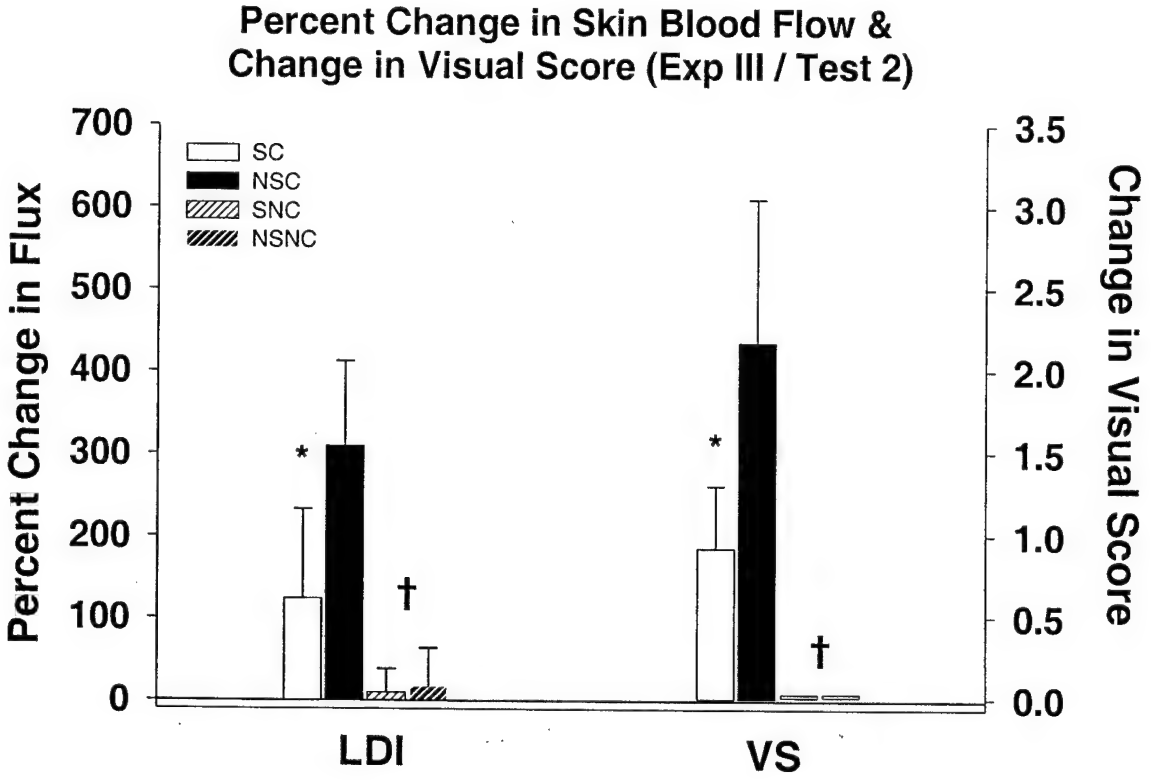
VS Pre

Subject #	SC	NSC	SNC	NSNC
11	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0
16	0.0	0.0	0.0	0.0
Mean	**0.0**	**0.0**	**0.0**	**0.0**
SD	**0.0**	**0.0**	**0.0**	**0.0**

VS Post

Subject #	SC	NSC	SNC	NSNC
11	1.0	1.0	0.0	0.0
12	1.0	2.0	0.0	0.0
13	1.5	3.5	0.0	0.0
14	0.5	1.5	0.0	0.0
15	1.0	2.5	0.0	0.0
16	0.5	2.5	0.0	0.0
Mean	**0.9***	**2.2**	**0.0†**	**0.0†**
SD	**0.4**	**0.9**	**0.0**	**0.0**

Figure 4. The mean (±SD) percentage change in flux from LDI scans at baseline and after Mnic challenge (right ordinate) and for the visual scores after Mnic challenge (left ordinate) for Experiment III, Test 2 (16 h). Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. * NSC > SC ($P \leq 0.001$); † SC > NSC, NSNC ($P \leq 0.001$).



Because we observed that SERPACWA did not provide complete protection at 16 h, we tested its protection at 12 h of wear (Experiment III, Test 3). In this test, SERPACWA again provided skin protection as measured by LDI flux because there was a significant difference between the SERPACWA-treated and Mnic challenged site and the no SERPACWA treatment, Mnic-challenged site ($P \leq 0.001$, Table 4). Again we observed a significant difference in flux between the SERPACWA-treated, Mnic-challenged site and the SERPACWA-treated, no Mnic challenge site ($P \leq 0.001$). This significant difference indicated that the protection provided by SERPACWA was not complete after 12 h of wear. The calculated protection as a percentage change in flux was improved at 12 h of wear to 66%, as shown in Figure 5. As seen for the prior two tests, the visual scores showed similar results as the flux data (Table 4). Figure 4 also shows the change in visual scores.

Table 4. Skin perfusion (LDI) and visual score (VS) data for each subject for Experiment III, Test 3 (12 h). Data listed under the headings LDI Pre and VS Pre was determined before application of SERPACWA. Data listed under the headings LDI Post and VS Post was determined after 12 h of wear. Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. *NSC > SC ($P \leq 0.001$); and †SC > SNC & NSNC ($P \leq 0.001$).

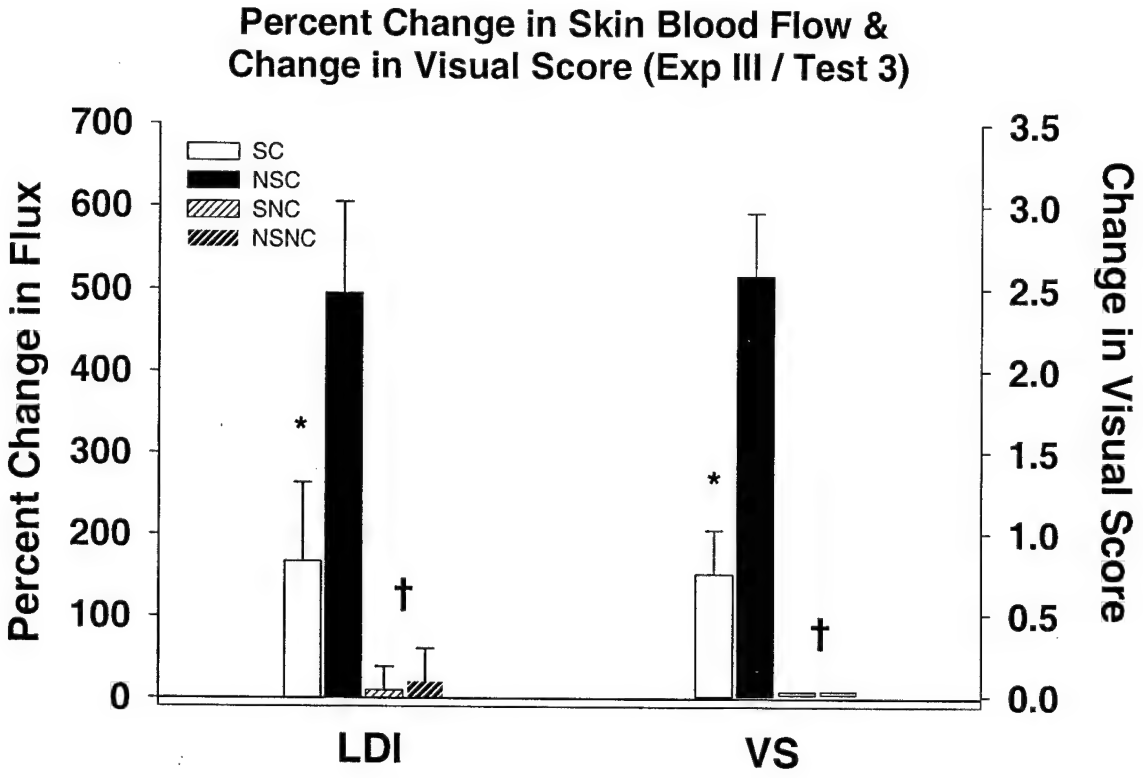
LDI Pre				
Subject #	**SC**	**NSC**	**SNC**	**NSNC**
11	49.8	69.1	51.4	71.4
12	94.5	65.7	69	67.9
14	57.2	52.2	43.6	72.5
15	38	34.3	32.3	30.1
16	120.3	53.9	62.4	63.4
19	53.2	56.7	56.5	50.1
Mean	**68.8**	**55.3**	**52.5**	**59.2**
SD	**31.6**	**12.3**	**13.2**	**16.4**

LDI Post				
Subject #	**SC**	**NSC**	**SNC**	**NSNC**
11	98.1	333.9	35.3	40.6
12	190	344.2	61	89
14	211.6	298.8	57.6	117.5
15	130.3	268.1	42.7	45
16	176.5	358.9	61.3	57.3
19	183.8	309	78.4	68.2
Mean	**165.1***	**318.8**	**56.1†**	**69.6†**
SD	**42.3**	**33.3**	**15.2**	**29.2**

VS Pre				
Subject #	**SC**	**NSC**	**SNC**	**NSNC**
11	0	0	0	0
12	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
19	0	0	0	0
Mean	**0.0**	**0.0**	**0.0**	**0.0**
SD	**0.0**	**0.0**	**0.0**	**0.0**

VS Post				
Subject #	**SC**	**NSC**	**SNC**	**NSNC**
11	1	3	0	0
12	0.5	2	0	0
14	0.5	2.5	0	0
15	1	2.5	0	0
16	0.5	3	0	0
19	1	2.5	0	0
Mean	**0.8***	**2.6**	**0.0†**	**0.0†**
SD	**0.3**	**0.4**	**0.0**	**0.0**

Figure 5. The mean (±SD) percentage change in flux from LDI scans at baseline and after Mnic challenge (right ordinate) and for the visual scores after Mnic challenge (left ordinate) for Experiment III, Test 3 (12 h). Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. * NSC > SC ($P \leq 0.001$); † SC > NSC, NSNC ($P \leq 0.001$).



The fourth test of Experiment III was done after the Sponsor and Combat Developer requested that a 24 h test of SERPACWA's efficacy be done, even though its protection was not complete after 16 h. There were no significant differences among any of the test sites (SERPACWA-treated, Mnic-challenged site vs. no SERPACWA treatment, Mnic challenged site vs. SERPACWA-treated, no Mnic challenge site vs. no SERPACWA treatment, no Mnic challenge site [$P = 0.20$, Table 5]). In contrast, visual scores following Mnic-challenge were significantly lower on the SERPACWA-treated arm (0.35±0.42) compared to the SERPACWA-untreated control arm (1.0 ±0.62, $P = 0.007$, Table 5). Figure 6 shows the calculated protection as a percentage change in flux and the change in visual scores.

Table 5. Skin perfusion (LDI) and visual score (VS) data for each subject for Experiment III, Test 4. Data listed under the headings LDI Pre and VS Pre was determined before application of SERPACWA. Data listed under the headings LDI Post and VS Post was determined after 24 h of wear. Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. #The visual score for NSC at 24 h was significantly different from the other 3 treatments (P=0.007).

LDI Pre

Subject #	SC	NSC	SNC	NSNC
7	28.7	32.2	34.7	51.2
20	53.3	60.8	55.2	45.3
22	28.4	33	29.3	44.4
23	30.3	34.5	30.2	28.2
24	117.4	93.7	69.9	64.3
Mean	**51.6**	**50.8**	**43.9**	**46.7**
SD	**38.2**	**26.8**	**18.0**	**13.0**

LDI Post

Subject #	SC	NSC	SNC	NSNC
7	50.1	71.2	68.6	66.3
20	52.4	71.1	61.1	59.3
22	44.8	93.4	52.9	89.7
23	59.1	52.7	63.9	44.7
24	259.9	365.9	65.7	84.1
Mean	**93.3**	**130.9**	**62.4**	**68.8**
SD	**93.3**	**132.2**	**6.0**	**18.4**

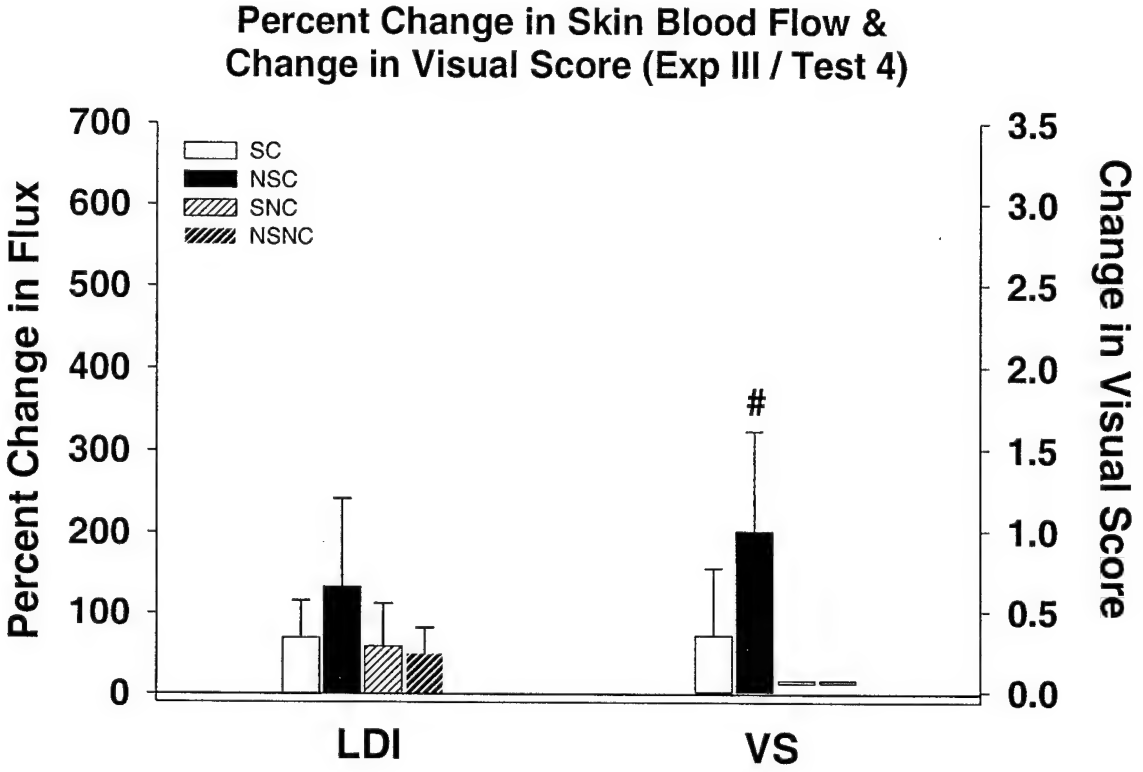
VS Pre

Subject #	SC	NSC	SNC	NSNC
7	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.5
23	0.0	0.0	0.0	0.0
24	0.0	0.0	0.0	0.0
Mean	**0.0**	**0.0**	**0.0**	**0.1**
SD	**0.0**	**0.0**	**0.0**	**0.2**

VS Post

Subject #	SC	NSC	SNC	NSNC
7	0.0	0.5	0.0	0.0
20	0.25	1.0	0.0	0.0
22	0.0	0.5	0.0	0.0
23	0.5	1.0	0.0	0.0
24	1.0	2.0	0.0	0.0
Mean	**0.35**	**1.0#**	**0.0**	**0.0**
SD	**0.42**	**0.61**	**0.0**	**0.0**

Figure 6. The mean (±SD) percentage change in flux from LDI scans at baseline and after Mnic challenge (right ordinate) and for the visual scores after Mnic challenge (left ordinate) for Experiment III, Test 4 (24 h). Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. # NSC >SC, NSC, NSNC and SC = NSC, NSNC ($P \leq 0.01$).



Experiment IV was a test of the skin protection provided by SERPACWA when it was re-applied after 8 h of wear and tested at 24 h after the initial application of SERPACWA. SERPACWA provided skin protection as measured by LDI flux as shown by the significant difference between the SERPACWA-treated, Mnic-challenged site and the no SERPACWA treatment, Mnic-challenged site ($P \leq 0.001$, Table 6). There was a significant difference in flux between the SERPACWA-treated, Mnic-challenged site and the SERPACWA-treated, no Mnic challenge site ($P \leq 0.001$). This significant difference indicated that the protection provided by SERPACWA, although not complete after 24 h of wear (with re-application at 8 h), was 58% when protection was calculated as a

percentage change in flux (Figure 7). The visual scores showed similar results as the flux data (Table 6). Figure 6 also shows the change in visual scores.

Table 6. Skin perfusion (LDI) and visual score (VS) data for each subject for Experiment IV (24 h of wear with reapplication of SERPACWA after 8 h). Data listed under the headings LDI Pre and VS Pre was determined before application of SERPACWA. Data listed under the headings LDI Post and VS Post were determined for 24 h of wear which included re-application of SERPACWA 8 h after the initial application. Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. *NSC > SC ($P \leq 0.001$); and †SC > SNC & NSNC ($P \leq 0.001$).

LDI Pre

Subject #	SC	NSC	SNC	NSNC
12	52.4	81.7	68.7	56.8
13	23.4	29.2	27.3	25
14	58.6	70	51.8	52.1
16	65.9	72.1	54.3	61.6
17	50.8	50.7	41.7	36.3
19	63.1	45.5	63.1	57
Mean	**52.4**	**58.2**	**51.2**	**48.1**
SD	**15.4**	**19.7**	**15.0**	**14.3**

LDI Post

Subject #	SC	NSC	SNC	NSNC
12	216.3	371.9	70.8	85.1
13	58.7	250.1	23.9	25.4
14	45.6	276.6	33.8	55.7
16	282.5	309.7	41.6	53.1
17	156.3	334.4	33.2	39.5
19	165	241.7	44.8	53.3
Mean	**154.1***	**297.4**	**41.4†**	**52.0†**
SD	**90.9**	**50.7**	**16.2**	**19.9**

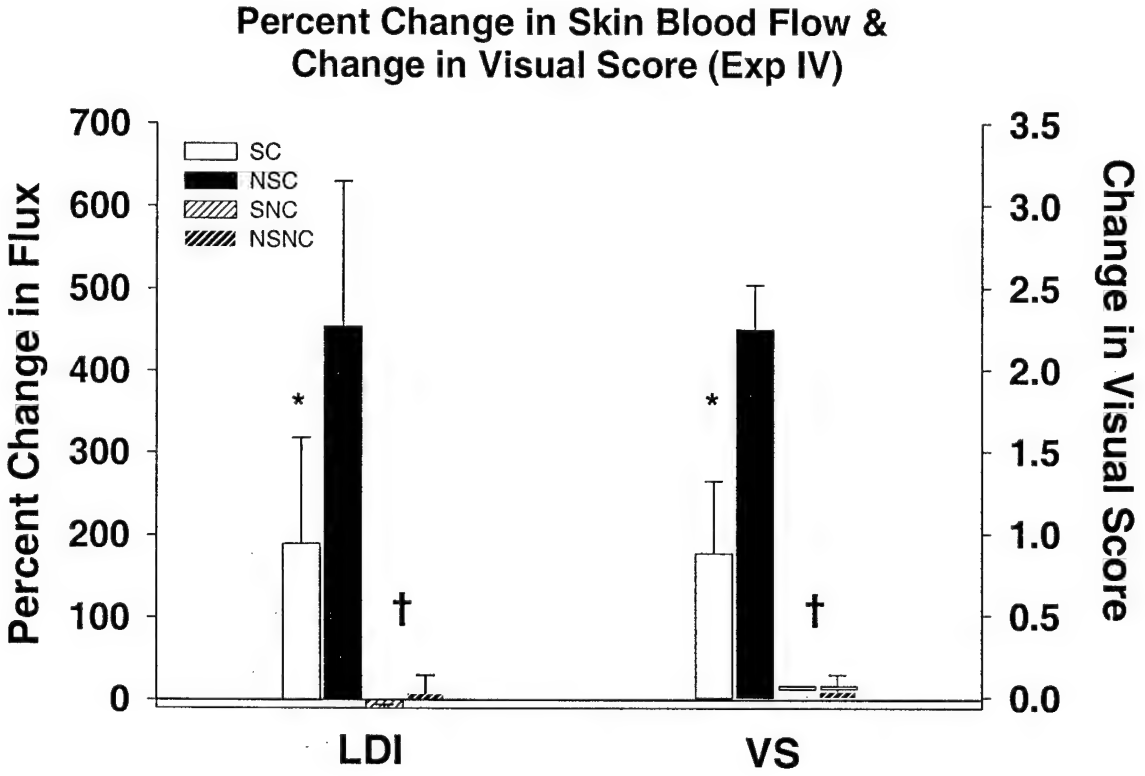
VS Pre

Subject #	SC	NSC	SNC	NSNC
12	0.0	0.0	0.0	0.0
13	0.0	0.0	0.0	0.0
14	0.0	0.5	0.0	0.5
16	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	-
19	0.0	0.0	0.0	-
Mean	**0.0**	**0.1**	**0.0**	**0.1**
SD	**0.0**	**0.2**	**0.0**	**0.3**

VS Post

Subject #	SC	NSC	SNC	NSNC
12	1.0	2.0	0.0	0.0
13	-	2.0	0.0	0.0
14	0.5	2.5	0.0	0.0
16	1.5	2.5	0.0	0.0
17	1.0	2.5	0.0	0.0
19	1.0	2.0	0.0	-
Mean	**1.0***	**2.3**	**0.0†**	**0.0†**
SD	**0.4**	**0.3**	**0.0**	**0.0**

Figure 7. The mean (±SD) percentage change in flux from LDI scans at baseline and after Mnic challenge (right ordinate) and for the visual scores after Mnic challenge (left ordinate) for Experiment IV (24 h test but with SERPACWA reapplied at 8 h. Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. * NSC > SC ($P \leq 0.001$); † SC > NSC, NSNC ($P \leq 0.001$).



DISCUSSION

During the NDA approval process for SERPACWA, the issue of greatest concern raised by the FDA about SERPACWA efficacy was its durability. The human research study "Skin Reduction Paste Against Chemical Warfare Agents (SERPACWA): Durability and Other Issues Related to Its Use" was designed to determine approximately how long SERPACWA provided complete protection against a skin vasodilatory drug, methyl nicotinate (17). The study was also designed to measure the degree of skin protection that SERPACWA provided for up to 24 h of wear. Finally, the study was designed to measure the improvement in skin protection afforded by re-application of SERPACWA at the time when it still provided complete protection. In the study protocol, we determined that the measurement of skin perfusion, as measured by LDI, would be the primary criterion for determining the degree of skin protection that

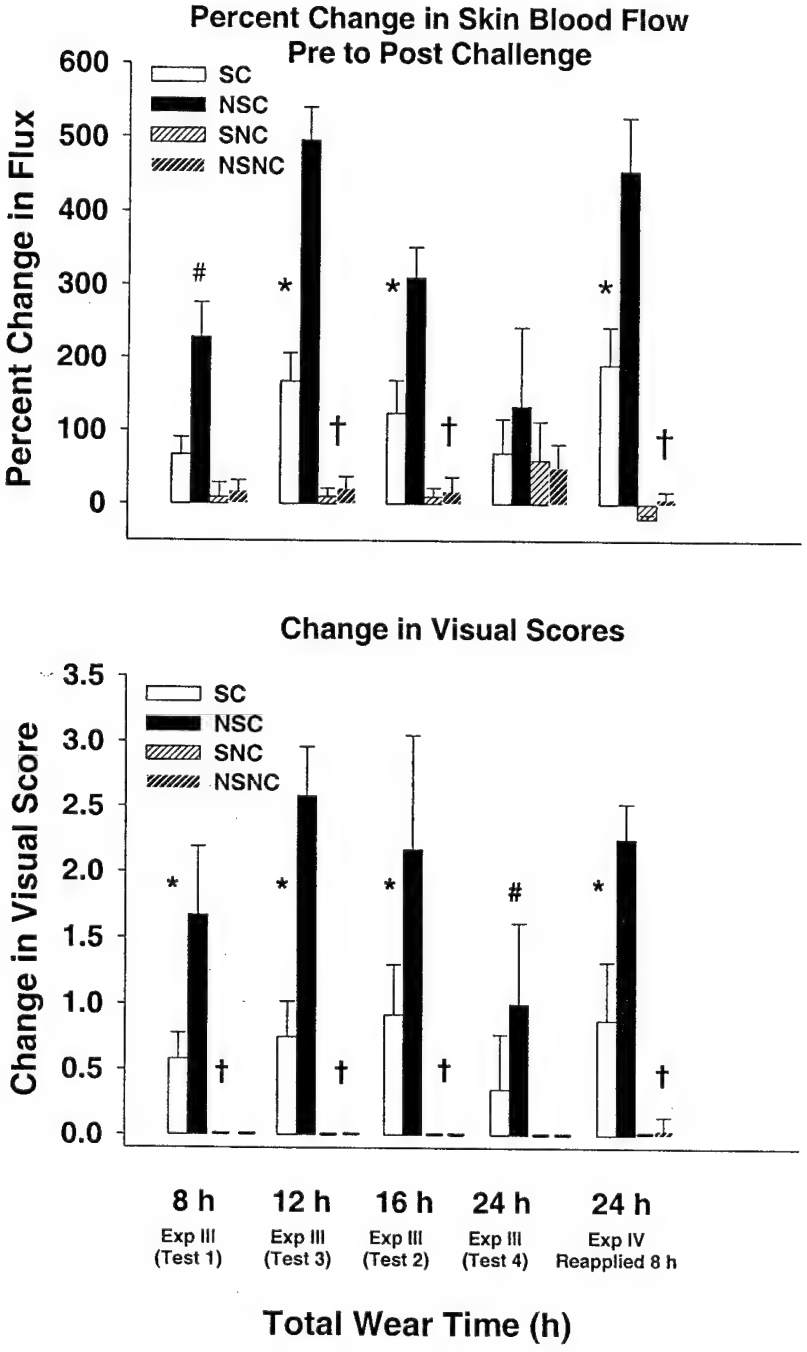
SERPACWA afforded. We chose to use visual scoring as a corroborating measurement for LDI.

Experiment III provided clear and convincing evidence that a film of SERPACWA having an approximate thickness of only 0.1 mm significantly reduced skin exposure to a Mnic challenge that otherwise induced significant vasodilation. When SERPACWA was applied once and worn under field relevant garments for 8 h, it significantly protected the skin (Table 2 and Figure 3, $P \leq 0.001$). This high degree of skin protection, as measured by LDI flux, met the definition of complete skin protection when SERPACWA was worn for 8 h. In addition, SERPACWA significantly protected the skin against Mnic challenge when LDI flux was measured at 12 (Table 4 and Figure 5; $P \leq 0.001$) and 16 h (Table 3 and Figure 4, $P \leq 0.001$). Although we classified this was not complete protection, SERPACWA still afforded significant protection against Mnic challenge based on percentage change in perfusion as measured by LDI. SERPACWA provided 66% protection at 12 h ($P \leq 0.001$), and 61% protection at 16 h ($P \leq 0.001$).

The test to determine SERPACWA protection at 24 h was inconclusive based on the LDI data (Table 5 and Figure 6). It is important to take a closer look at the skin perfusion data for Experiment III, Test 4, because the response to the Mnic challenge was clearly blunted, as can be seen in the LDI and VS data (Figure 8). Compared to the other tests, both the LDI and visual scores were greatly reduced following Mnic challenge when the skin was not protected with SERPACWA. The magnitude of this reduced skin response to Mnic challenge, as measured by LDI, was enough that the vasodilatory response to Mnic could not be detected statistically. Analysis of the LDI data shows that there was no difference in perfusion (percentage change from baseline) between the SERPACWA-treated (SC = 69.4 ± 46.3%) and SERPACWA-untreated control (NSC = 132.9 ± 108.8%) arms when challenged with Mnic ($p=0.20$). There was also no difference between the Mnic-challenged (SC) and unchallenged SERPACWA-treated (SNC) sites ($p=0.20$). In fact, none of the four treatment sites (SC, NSC, SNC, NSNC) exhibited a statistically significant difference from one another, preventing any interpretation whatsoever from LDI data. This finding indicated that the skin response to Mnic was greatly reduced from previous tests and had the result that Mnic was an ineffective stimulus in this test. In contrast, visual scores following methyl nicotinate challenge were significantly lower on the SERPACWA-treated arm (SC = 0.35±0.42) compared to the SERPACWA-untreated control arm (NSC = 1.0 ± 0.62, $p = 0.0007$).

The differences in visual scores indicated that although protection afforded by SERPACWA is reduced compared to shorter wear times, it is still significantly better than not having SERPACWA present. Further comparison of the sites on the SERPACWA-treated arm shows that the challenged site cannot be distinguished statistically from the SERPACWA treated, no Mnic challenge site. As interpreted from LDI data in previous experiments, this would indicate that SERPACWA provides complete protection. Although the interpretation that SERPACWA provides a reduced but still significant protection is consistent with results from previous experiments; the

Figure 8. The mean (±SD) percentage change in flux from LDI scans at baseline and after Mnic challenge (top) and for the visual scores after Mnic challenge (bottom) for all tests in Experiments III and IV. Abbreviations: SC: SERPACWA treatment, challenged with 5 mMol Mnic; NSC: no SERPACWA treatment, challenged with 5 mMol Mnic; SNC: SERPACWA treatment, not challenged with Mnic; and NSNC: no SERPACWA treatment, not challenged with Mnic. # NSC >SC, NSC, NSNC and SC = NSC, NSNC ($P \leq 0.001$); * NSC > SC ($P \leq 0.001$); † SC > NSC, NSNC ($P \leq 0.001$).



results indicating complete protection is not. As in the case of the LDI data, we believe that the reduced response to the challenge agent is responsible for a lower dynamic range for both SERPACWA-treated and untreated sites. For example, the average visual score for no SERPACWA, Mnic-challenged sites for the previous three experiments was between 1.7 - 2.6 and only 1.0 in the present experiment. In addition, there was greater variability in skin perfusion data during the control tests when there was no Mnic challenge. These observations, considered together, make it clear that the strength of the Mnic stimulus was not nearly the same magnitude as was observed in previous tests.

There are several possible reasons why there was a reduced response to Mnic challenge and increased individual variation in skin perfusion. It is likely that the Mnic challenge was less potent in Experiment III, Test 4, because the vascular properties of the skin, when the measurements were recorded (in the morning between 0836 –1106 h), were different than for the other tests. Skin perfusion and visual score measurements made following Mnic challenge for all the other tests were in the afternoon (Experiment III, Test 1 = 1645 h –1800 h; Experiment IV = 1312 h-1427h), evening (Experiment III, Test 3 = 2050 h – 2206 h) or early morning (Experiment III, Test 2 =0056 h- 0214 h). Reports of circadian variation in vascular tone indicated that skin vasculature was relatively constricted in the morning hours (22, 26). This may account for the diminished response to Mnic, as seen in both the LDI and the VS data.

Another reason for the reduced skin response to the Mnic challenge may simply be due to subject heterogeneity. The subject population for the present experiment (5 evaluable subjects) was heterogeneous: 3 female African-Americans, 1 Caucasian with tan skin, and 1 Caucasian with freckled, pink skin. This variation in skin types among a population of only 5 subjects may have led to the high standard deviation in skin perfusion within all treatment sites. Subject groups of previously performed experiments were less heterogeneous in ethnicity (Table 1).

There was also greater inter-individual variation in the LDI data in Experiment III, Test 4, than in previous tests (Table 5 vs. Tables 2-4 and 6). It may be that vascular responsiveness of the skin is more variable in the morning than other times of day. For example, there may have been a high degree of vasoconstrictor tone in the cutaneous vasculature of most individuals when tested, while the morning increase in vasoconstrictor tone may have abated in one of the subjects. S7, S20, S22, and S23 had a much smaller response to Mnic challenge than did S24 (Table 5). This interpretation seems appropriate given that S24 was studied at the latest time in the morning. The flux measured at the no SERPACWA, Mnic-challenged skin test site during the LDI scan following Mnic challenge for S24 was a similar magnitude (Table 5) to other individuals in Tests 2 and 3 (Tables 3 and 4). This observation shows that Mnic itself did induce an expected vasodilatory response in this individual, so it is unlikely that the Mnic solution was responsible for the low vasodilatory response in Test 4. Individual variation in LDI data might also be due to a technical problem with the laser-Doppler instrument that caused a brief delay on the morning of the post-challenge

measurement. We restored operation by executing instructions from the technical representative, and the instrument calibration was within range. We do not believe that the instrument contributed to the variation in the data, however, because its pre- and post-test calibrations were within acceptable range for the post-challenge scan. The mean and standard deviation of the control site LDI data (SNC and NSNC) for Experiment III, Test 4 (Table 5) were not noticeably different than other tests (Tables 2-4 and 6).

It is difficult to interpret the results from Experiment III (Test 4). On one hand, the absence of a significant response to Mnic challenge on even those sites not treated with SERPACWA, as measured by LDI, thwarts evaluation of SERPACWA's efficacy at 24 h of wear. It appears that circadian variation in skin blood flow and heterogeneity of skin types among subjects may have resulted in a lower average perfusion response with a wider variability than was observed in this study with previous groups of subjects. The effect of these factors on the results was that even the sites not treated with SERPACWA showed a response to Mnic that was not significantly different from the control site (no Mnic treatment, no SERPACWA treatment). On the other hand, one would conclude from the results of visual scoring that even after 24 h of wear-time, SERPACWA significantly reduced the response to the challenge agent compared to sites that were challenged but not treated with SERPACWA.

Analysis of this experiment is confounded by the lack of meaningful data from laser-Doppler measurements, selected as the primary end point prior to protocol approval by the FDA. Although visual scoring has proven to be reliable and accurate over decades of use, LDI was deemed to be less subject to bias. But in the present experiment, LDI was unable to distinguish among any of the experimental sites. Whether this was due to heterogeneity of skin types and/or to a circadian variation in blood flow, the end result is the same: the results do not permit evaluation of SERPACWA efficacy. Fortunately, a second method, visual scoring, was also employed. These data were collected in this and previous experiments to corroborate with the LDI results, with a clear distinction being given to LDI in the event that results between the two should conflict. In previous experiments that tested durability at shorter wear times, laser-Doppler and visual scoring data correlated well (Figure 8). However, in the present experiment, results of the two methodologies conflict; but because LDI data did not show a statistically significant skin response to Mnic challenge, the LDI and VS data cannot be compared. Results from visual scores can be evaluated while results from LDI cannot. Therefore, although the protocol states that visual scores would be used for corroboration only, we submit that realistic conclusions can be made using visual scores, since it is a historically proven technique (1, 6, 10, 19, 20) and it has correlated with well LDI in the current research (Figure 8; (17)). Using visual scores, even after 24-h of wear-time, SERPACWA significantly reduced the response to the challenge agent compared to sites that were not treated with SERPACWA and challenged. From visual score data we conclude that SERPACWA has a small but significant protective effect after 24-hours of continuous wear. As stated earlier, we recommend performing the experiment again with a larger subject population, where circadian-induced changes in skin blood flow is controlled by use of a challenge schedule that matches one of the previous wear-time experiments.

In Experiment III we determined that SERPACWA provided complete skin protection for 8 h of wear. Experiment IV provided clear and convincing evidence that SERPACWA also significantly protected the skin when it was re-applied to skin after 8 h and tested for protection at 24 h of wear, with the 24 h period starting at initial application. SERPACWA provided 58% protection ($P \leq 0.001$, Table 6) when it was re-applied after 8 h of wear and tested at 24 h after initial application. From these results one might infer that re-application of SERPACWA at the closure sites of chemical protective clothing should be done after 8 h of wear, if possible. If it is not, SERPACWA still significantly protects the skin from a vasodilatory challenge. Note that SERPACWA durability was determined when the subjects wearing it were not actively exercising. In some cases, in particular during Experiment III, Tests 1-3 and Experiment IV, the subjects did sweat during the time of wear. It is known that SERPACWA protects the skin of subjects when they are actively sweating (6). However, the current study did not address the issue of whether or not SERPACWA durability would be similar under conditions in which the subjects were actively sweating.

CONCLUSIONS

SERPACWA is a durable skin exposure reduction paste when worn on forearm skin under chemical protective garments by soldiers who are not exercising. A film of SERPACWA having an approximate thickness of only 0.1 mm very significantly reduced skin exposure to Mnic challenges that otherwise induced significant vasodilation. When it is worn for 8 h on forearm skin, SERPACWA completely (as defined above) protects the skin after it is challenged with Mnic. The data from the current tests provide clear and convincing evidence that SERPACWA possesses significant skin protection when applied once and worn under field relevant garments for 12 and 16 h. SERPACWA also significantly protected the skin when it was re-applied to skin after 8 h and tested for protection at 24 h of wear. The LDI data for the 24 h continuous wear test (Experiment III, Test 4) were inconclusive. The VS data for this test indicate that SERPACWA partially protected the skin (65%) after 24 h wear.

RECOMMENDATIONS

It is recommended that SERPACWA be applied to the skin at closure sites of chemical protective clothing to provide additional protection for up to 24 h against threat agents. When it is possible to safely remove chemical protective clothing, SERPACWA should be reapplied to the skin after 8 h of wear to optimize skin protection.

It is also recommended that further research be done to determine whether prolonged sweating will reduce the durability of SERPACWA.

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